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<p>(21) International Application Number: PCT/US88/02760 (22) International Filing Date: 15 August 1988 (15.08.88) (31) Priority Application Number: 035,627 (32) Priority Date: 17 August 1987 (17.08.87) (33) Priority Country: US (71) Applicant: THE REGENTS OF THE UNIVERSITY OF CALIFORNIA [US/US]; 2199 Addison Street, Berkley, CA 94720 (US). (72) Inventors: PARDRIDGE, William, M. : 1180 Tellem Drive, Pacific Palisades, CA 90272 (US). SCHIMMEL, Paul, R. : 31 Solomon Pierce Road, Lexington, MA 02173 (US).</p>	<p>(74) Agents: OLDENKAMP, David, J. et al.; Poms, Smith, Lande & Rose, 2121 Avenue of the Stars, Suite 1400, Los Angeles, CA 90067 (US). (81) Designated States: AT (European patent), AU, BE (European patent), BR, CH (European patent), DE (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent), SU. Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p>	
<p>(54) Title: CATIONIZED ANTIBODIES FOR DELIVERY THROUGH THE BLOOD-BRAIN BARRIER</p> <p>(57) Abstract</p> <p>The rate of transcytosis of antibodies across the blood-brain barrier is increased by cationizing the antibodies to provide cationized antibodies having an isoelectric point of between about 8.0 to 11.0. The increased rates of transport across the blood-brain barrier makes such cationized antibodies useful for both neurodiagnostic and neuropharmaceutical purposes. Methods for preparing such cationized antibodies are disclosed.</p>		

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CATIONIZED ANTIBODIES FOR
DELIVERY THROUGH THE BLOOD-BRAIN BARRIER

Background of the Invention

5 The present invention relates generally to the use
of antibodies for treatment and diagnosis of neurologi-
cal diseases. More particularly, the present invention
deals with the modification of antibodies so that they
can be delivered through the blood-brain barrier by
10 transcytosis.

This invention was made with Government support
under Grant No.: DK 25744 with the National Institutes
of Health and the University of California. The
Government has certain rights in this invention.

15 Antibodies in general, and especially monoclonal
antibodies, are widely used in diagnostic tests as a
means for detecting the presence of specific antigens.
Enzyme linked immunoassay and radioimmunoassay are
common diagnostic techniques which utilize antibodies
20 and detect antigens in vivo. Antigens may also be
detected in vitro by administering radiolabelled
antibodies to a living subject followed by external
detection of radiolabelled antibody sequestered by a
particular organ bearing the respective antigen.
25 Antibodies have also been used widely in the treatment
of viral infections and other diseases. However, the
use of antibodies in either the treatment or diagnosis
of neurological diseases has been very limited because
most antibodies are not capable of traversing the blood-
30 brain barrier (BBB) and entering the brain.

The vertebrate brain has a unique capillary system which is unlike that in any other organ in the body. The unique capillary system has morphologic characteristics which make up the blood-brain barrier. The blood-brain barrier acts as a system wide cellular membrane which separates the brain interstitial space from the blood. The unique morphologic characteristics of the brain capillaries which make up the blood-brain barrier are: (a) epithelial-like high resistance tight junctions which literally cement all endothelia of brain capillaries together, and (b) scanty pinocytosis or transendothelial channels, which are abundant in endothelia of peripheral organs. Due to the unique characteristics of the blood-brain barrier, antibodies that readily gain access to other tissues in the body are barred from entry into the brain or their rates of entry are very low.

Few strategies have been developed for introducing these antibodies into the brain which otherwise would not cross the blood-brain barrier. The most commonly used strategy involves an invasive procedure where the antibody is delivered directly into the brain. The most common procedure is the implantation of a catheter into the ventricular system to bypass the blood-brain barrier and deliver the antibody directly to the brain. Such a procedure has been used in treating echovirus encephalitis (Erlendsson et al., Successful Reversal of Echovirus Encephalitis in X-linked Hypogammablobulinemia by Intraventricular Administration of Immunoglobulin. 1985. New England Journal of Medicine. Vol. 312, No. 6. pages 351-353).

Although invasive procedures, such as the one described above, for the direct delivery of antibodies to the brain ventricles have experienced some success, they are not entirely satisfactory because they do not deliver the antibodies to the structures deep within the

brain. Further, the invasive procedures are potentially harmful to the patient. Accordingly, there presently is a need to provide an improved method for delivering antibodies across the blood-brain barrier and into the
5 brain for both diagnostic and therapeutic purposes.

Summary of the Invention

In accordance with the present invention, a method is provided for introducing antibodies directly across
10 the blood-brain barrier by transcytosis. The invention is based upon the discovery that cationized antibodies cross the blood-brain barrier at much higher rates than normal non-cationized antibodies.

The effectiveness of antibodies for both neurodiag-
15 nostic and neuropharmaceutical purposes is increased by cationizing the antibodies to provide cationized antibodies having an isoelectric point (pI) of between about 8.0 to 11.0. These highly basic antibodies cross the blood-brain barrier at rates which are much higher than
20 the transcytosis rates for normal acid and neutral antibodies which typically have isoelectric points in the range of 5 to 6. This provides an effective means for delivering antibodies into the brain by transcytosis rather than by the previously used direct invasive
25 methods.

The cationized antibodies in accordance with the present invention are prepared by treating a given monoclonal or polyclonal antibody with a cationization agent such as hexamethylenediamine. The cationization
30 agent replaces surface carboxyl groups on the antibody with a more basic group, such as a primary amine group in the case of hexamethylenediamine and related amine compounds. The amount of cationization agent and reaction conditions are controlled so that the resulting
35 cationized antibody has an isoelectric point of between about 8.0 to 11.0 and preferably between about 8.0 to

9.0.

As one feature of the present invention, the immunoreactivity of the antibodies is preserved during cationization by first reacting the antibody with an excess of a corresponding antigen to block the immunoreactive sites on the antibody. These blocked immunoreactive sites are unreactive during the subsequent cationization steps. The antigens are then decoupled from the cationized antibodies after the cationization step to thereby reactivate the blocked immunoreactive sites.

The cationization of antibodies in accordance with the present invention is useful whenever it is necessary to introduce an antibody into the brain. Both neurodiagnostic and neurotherapeutic uses for antibodies is contemplated. Particular diagnostic uses include diagnosis of Alzheimer's disease, brain tumors or any other diagnostic use where a labeled or tagged antibody is introduced into the brain for reaction with and detection of specific antigens. Therapeutic uses include treatment of viral infections of the brain or other diseased conditions where introduction of an antibody into the brain is required to treat the disease.

The above discussed and many other features and attendant advantages of the present invention will become apparent as the invention becomes better understood by reference to the following detailed description.

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Brief Description of the Drawings

Figure 1 is a graph showing the increase in uptake of cationized IgG by brain capillaries at both 4°C and 37°C. The results are expressed as percent uptake of IgG per milligram of IgG.

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Figure 2 is a graph which also shows the increase

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in uptake of cationized IgG by brain capillaries. The results are expressed as percent uptake of IgG versus amount of brain capillaries.

Figure 3 is a graph showing the uptake of cation-
5 ized IgG plotted versus the concentration of either native IgG or cationized IgG.

Detailed Description of the Invention

The present invention involves the transport of
10 antibodies through the brain capillary wall, ie. the blood-brain barrier (BBB). The nature of the blood-brain barrier and problems associated with transport of peptides and proteins therethrough is set forth in "Receptor-Mediated Peptide Transport through the Blood-
15 Brain Barrier" (W.M. Pardridge, Endocrine Reviews, Vol. 7, No. 3, August 1986, pages 314 -330), the contents of which is hereby incorporated by reference.

The present invention has wide application to any antibody which is useful in diagnosing or treating brain
20 disorders. Antibodies in general do not readily cross the blood-brain barrier. This is due to the acidic or neutral character of antibodies. It was discovered that the uptake or transport of antibodies into the brain can be greatly increased by cationizing the antibodies to
25 form cationized antibodies having an isoelectric point of between about 8.0 to 11.0.

Antibodies are proteins which have both positive and negative charges with the number of each depending upon the pH of the antibody solution. The pH at which
30 the positive and negative charges are equal is called the "isoelectric point" (pI). Techniques for measuring the pI of a given antibody or protein are well known and generally involve isoelectric focusing according to conventional electrophoresis procedure. As previously
35 mentioned, most antibodies have an isoelectric point of between about 5 to 6.

The relatively low isoelectric point of antibodies is due to the presence of carboxyl groups on the surface of the antibodies. The present invention involves substituting basic groups in place of a sufficient
5 number of surface carboxyl groups to increase the pI of the antibody to between about 8.0 to 11.0. Isoelectric points of between about 8.0 to 9.0 are preferred with isoelectric points of around 8.5 being especially preferred. The degree of cationization should be as
10 high as possible without causing the antibody to form into aggregates. Higher pI's are preferred because the rate of transport of the antibody across the blood-brain barrier increases with increasing pI. However, this must be offset by the increasing possibility of
15 antibody aggregate formation at higher levels of cationization.

Cationization of the antibody can be carried out according to any of the known procedures for displacing surface carboxyl groups on proteins with basic cations.
20 Preferred cationization agents include amine compounds such as hexamethylenediamine and related amine compounds. Hexamethylenediamine is the preferred cationization agent because it is widely available and the techniques for its use in cationizing proteins are well
25 known. The amount of cationizing agent and the conditions for reaction with the antibody can be varied so long as the final cationized antibody has a pI within the above-mentioned range required for blood-brain barrier transport.

30 The particular antibodies which can be used are virtually unlimited, provided that they have some diagnostic or therapeutic use in connection with the brain. Monoclonal antibodies are preferred because of their increased diagnostic or therapeutic potential.
35 Typical antibodies which can be cationized for blood-brain barrier transcytosis are antibodies to one or more

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of the antigenic portions of peptides specific to Alzheimer's disease (Pardridge, W.M. et al., Amyloid Angiopathy of Alzheimer's Disease: Amino Acid Composition and Partial Sequence of a 4,200 - Dalton Peptide Isolated from Cortical Microvessels, Journal of Neurochemistry, 1987, pages 001 - 008). Antibodies to such specific peptides can be tagged with a radioactive tracer or other identifier and then cationized to a pI of 8.5 with hexamethylenediamine. The resulting tagged and cationized antibody can then be administered intravenously to the patient using a suitable pharmaceutically acceptable carrier solution. The tagged and cationized antibody will cross the blood-brain barrier and enter the brain where it will bind to any of the peptides which are unique to Alzheimer's disease. Detection of the bond tagged and cationized antibody which is bound to the specific peptides is then performed by convention of neuroimaging techniques, such as external detection nuclide counting.

Other diagnostic antibodies which can be cationized to provide entry into the brain include antibodies for use in detecting various types of brain tumors. For example, monoclonal antibodies to tumor specific proteins such as glial fibrillary acidic protein (GFAP) can be prepared by conventional and well known techniques for monoclonal antibody preparation. Antibodies to human DR antigen and human immunodeficiency virus HIV antigen are other examples.

The resulting monoclonal antibodies are treated with hexamethylenediamine or other cationization agents to increase the pI of the antibody to between about 8.0 to 11.0. The antibody can be tagged with a radioactive tracer prior to or after the cationization process. The resulting tumor specific cationized and tagged antibody is then administered to the patient intravenously for transport across the blood-brain barrier and binding to

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any tumor specific antigen. Detection of bound antibody is again accomplished by convention radionuclide scanning techniques.

5 Cationized antibodies for use in treating viral diseases such as AIDS or other disorders of the brain can also be prepared as set forth above. Once an antibody (preferably monoclonal) is prepared for a specific neurotropic virus or other infectious agent, the antibody is cationized to increase its pI to between
10 about 8.0 to 11.0. The antibody is then administered intramuscularly or intravenously to the patient. The antibody is typically administered as a solution of antibody in a suitable pharmaceutical carrier such as saline buffer. The doses of cationized antibody
15 administered for either diagnostic or therapeutic purposes will parallel the dosage levels established for non-cationized antibodies. Typical dosages range from 0.01 mg to 1 mg for diagnostic purposes and from 1 mg to 100 mg for therapeutic purposes.

20 Preferred antibodies include chimeric human antibody molecules designed to have reduced antigenicity, such as those antibodies having mouse antigen-binding domains with human constant region domains. Such chimeric antibodies have been disclosed by S. L. Morrison et al. (Chimeric Human Antibody Molecules: Mouse Antigen-binding Domains with Human Constant Region Domains, Proc. Nat'l. Acad. Sci. USA, November 1984, Vol. 81, pages 6851 - 6855).

Although hexamethylenediamine is the preferred
30 compound for use in cationizing antibodies, other cationizing agents are possible. For example, ethylene diamine, N,N-dimethyl-1,3-propanediamine, or polylysine may be used. Cationization is catalyzed by carboxyl activation using N-ethyl,N¹(3-dimethyl-aminopropyl)
35 carbodiimide hydrochloride (EDAC) using the method described by Hoare and Koshland (A Method for the

Quantitative Modification and Estimation of Carboxylic Acid Groups in Proteins. 1967. J. Biol. Chem. 342:2447-2453).

In order to prevent reductions in the immunore-
5 activity of an antibody during cationization, it is preferred that the antibody be pre-bound to the antigen of interest prior to cationization. This pre-binding with antigen effectively blocks the immunoreactive sites on the antibody and prevents them from being cationized.
10 After cationization is complete and the pI of the antibody has been raised to the desired level between about 8.0 to 11.0, the cationized antibody is then treated to unbind the antigen from the antibody. The unbinding is accomplished according to well known
15 procedures where the antibody-antigen complex is treated with an acid to break the antibody-antigen bond. The antibody is then recovered by column chromatography or other conventional separation and recovery technique.

As an example of practice, bovine IgG was cation-
20 ized and tested against native bovine IgG as follows:

One gram of bovine immunoglobulin G was dissolved in 10 ml of water followed by dialysis at 4°C overnight against water. To this was added slowly 67 ml of 2 M hexamethylenediamine while stirring, and the pH was kept
25 at 7.8. Thirty minutes later, 1 g of EDAC was added and the pH was maintained at 7.8 and the solution was stirred at room temperature for 3-4 hours. The material was then dialyzed against 40 liters of water overnight at 4°C followed by evaporation to dryness the following
30 day.

The cationized antibody and native bovine antibody were then radiolabelled with ^3H -sodium borohydride using standard methods which have been described previously by Pardridge et al. (Absence of Albumin Receptor on Brain
35 Cappillaries In Vivo or In Vitro. 1985, Am. J. Physiol. 249:E264-E267; Chimeric Peptides as a Vehicle for

Peptide Pharmaceutical Delivery through the Blood-Brain Barrier. 1987, Biochem. Biophys. Res. Commun. 146:307-315). Bovine brain capillaries were isolated from fresh bovine brain and used as in vitro model system of the blood-brain barrier as reviewed by Pardridge, W.M. (Receptor-Mediated Peptide Transport through the Blood-Brain Barrier. 1986, Endocrine Reviews 7:314-330).

The results of the above tests with bovine IgG are set forth in Figures 1, 2 and 3. In Figure 1, the percent uptake per milligram protein of (^{125}I) cationized IgG or (^{125}I) native IgG at either 37°C or 4°C is plotted versus incubation time. The labeled cationized or native IgG was incubated with isolated bovine brain capillaries, which are used as an in vitro model system of blood-brain barrier transport. The results show that the cationization procedure increases the uptake of the IgG by nearly 50-fold, and that this is partially inhibited by cold temperatures. In Figure 2, the percent uptake of (^{125}I) cationized IgG or (^{125}I) native IgG is plotted versus the amount of bovine brain capillary protein content in micrograms per tube. There is approximately a 25-fold increase in the uptake of the IgG following cationization.

In Figure 3, the percent uptake of (^{125}I) cationized IgG per milligram protein of isolated bovine brain capillary is plotted versus the concentration of unlabeled cationized IgG or native IgG. The data show that the uptake of (^{125}I) cationized IgG is completely independent of concentration of native IgG through three log orders of magnitude in concentration. However, the presence of unlabeled cationized IgG stimulates the uptake in low concentrations and greatly depresses the uptake in high concentrations. The concentration of cationized IgG which causes 50% inhibition is approximately 2.5 mg/ml or approximately 15 μM cationized IgG.

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Further examples of practice are:

A monoclonal antibody may be prepared against a synthetic peptide corresponding to the 4200 Dalton amyloid peptide of Alzheimer's disease amyloid angiopathy (see Pardridge et al, Amyloid angiopathy of Alzheimer's disease: amino acid composition and partial sequence of a 4,200-Dalton peptide isolated from cortical microvessels, 1987 J. Neurochem 49.) This amyloid is deposited on the brain side of the BBB and, thus, a monoclonal antibody to the amyloid peptide cannot be used as a neuroimaging device unless the monoclonal antibody is transportable through the BBB. The monoclonal antibody to the synthetic amyloid peptide may be cationized using hexamethylenediamine and EDAC, in the presence of saturated concentrations of synthetic amyloid peptide (to protect the active antigen binding sites on the antibody), to an isoelectric point of between 8 - 11. The cationized antibody may then be separated from the antigen by gel filtration in the presence of 0.1 M glycine (pH = 2.5). The high molecular weight peak containing the cationized antibody is then neutralized to pH = 7.4 and is now suitable for radiolabelling using standard radionuclides such as technetium 99m or iodine-I¹³¹.

A monoclonal antibody to human GFAP may be prepared by isolating GFAP from human autopsy brain using standard techniques or by isolating recombinant human GFAP from either a bacterial or a eukaryotic expressing system. The monoclonal antibody to GFAP may then be cationized using hexamethylenediamine and EDAC in the presence of high concentrations of GFAP, followed by separation of cationized antibody from antigen as described above. The cationized monoclonal antibody to human GFAP may then be radiolabelled with technetium 99m or iodine-I¹³¹ or other conventional radionuclides. The final preparation is a radiolabelled antibody to GFAP

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that is transportable through the BBB and may be used as a neuroimaging device for early detection of brain glial tumors.

Another example is the preparation of mouse-human
5 chimeric antibody directed against the human DR-antigen. This mouse-human chimeric antibody may be cationized using hexamethylenediamine and EDAC in the presence of saturating concentrations of recombinant DR-antigen followed by separation of cationized antibody from the
10 free DR-antigen. The cationized human-mouse chimeric monoclonal antibody to the human DR-antigen may then be administered subcutaneously to subjects with demyelinating diseases, such as multiple sclerosis, that have an immune basis, and the pathogenesis of which may be
15 ameliorated by the administration of antibody against the DR-antigen. For example, Sriram and Steinman (Anti I-A Antibody Suppresses Active Encephalomyelitis: Treatment Model for Diseases Linked to IR Genes. 1983, J. Exp. Med. 158:1362-1367) have provided evidence that immune
20 linked demyelinating diseases may be treated by administration of antibody against the class II histocompatibility antigen.

Having thus described exemplary embodiments of the present invention, it should be noted by those skilled
25 in the art that the within disclosures are exemplary only and that various other alternatives, adaptations and modifications may be made within the scope of the present invention. Accordingly the present invention is not limited to the specific embodiments as illustrated
30 herein, but is only limited by the following claims.

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WHAT IS CLAIMED IS:

1. In a diagnostic composition comprising one or more antibodies for administration to an individual for neurodiagnostic purposes, said antibodies having a relatively low transfer rate across the blood-brain barrier, wherein the improvement comprises cationizing said antibodies to provide cationized antibodies having an isoelectric point of between about 8.0 and 11.0, said cationized antibodies having increased rates of transfer across said blood-brain barrier.
2. A diagnostic composition according to claim 1, wherein said antibodies are monoclonal antibodies.
3. A diagnostic composition according to claim 2 wherein the isoelectric point of said cationized antibodies is between about 8.0 to 9.0.
4. A diagnostic composition according to claim 1 wherein said antibody is an antibody to an Alzheimer's disease amyloid peptide.
5. A diagnostic composition according to claim 1 wherein said antibody is an antibody to one or more antigens present in GFAP protein.
6. In a neuropharmaceutical composition comprising one or more antibodies for administration to an individual for neurotherapeutic purposes, said antibodies having a relatively low transfer rate across the blood-brain barrier, wherein the improvement comprises cationizing said antibodies to provide cationized antibodies having an isoelectric point of between about 8.0 and 11.0, said cationized antibodies having increased rates of transfer across said blood-brain

10 barrier.

7. A neuropharmaceutical composition according to claim 6 wherein said antibodies are monoclonal antibodies.

8. A neuropharmaceutical composition according to claim 7 wherein the isoelectric point of said cationized antibodies is between about 8.0 to 9.0.

5 9. A neuropharmaceutical composition according to claim 6 wherein said antibody is an antibody to an antigen selected from the group consisting of amyloid peptide of Alzheimer's disease, human GFAP, human DR-antigen, or the human immunodeficiency virus (HIV).

10. A method for preparing an antibody having an increased transfer rate across the blood-brain barrier comprising the step of treating said antibody with an effective amount of a cationization agent to form a
5 cationized antibody having an isoelectric point of between about 8.0 to 11.0.

11. A method according to claim 10 wherein said antibody is a monoclonal antibody.

12. A method according to claim 10 including the additional steps of:

reacting said antibody with an antigen for said antibody to form an antibody having immunoreactive sites
5 blocked by said antigen prior to cationization of said antibody; and

treating the cationized antibody to remove said antigens therefrom to provide a cationized antibody having unblocked immunoreactive sites.

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13. A method according to claim 10 wherein said cationization agent is an amine cationization agent.

14. A method according to claim 13 wherein said amine cationization agent is hexamethylenediamine.

15. A method according to claim 10 wherein cationized antibodies having an isoelectric point of between about 8.0 to 9.0 are formed.

16. A method according to claim 14 wherein cationized antibodies having an isoelectric point of about 8.5 are formed.

17. A method according to claim 10 wherein said antibodies are antibodies for diagnostic uses.

18. A method according to claim 10 wherein said antibodies are antibodies for therapeutic uses.

19. A method according to claim 17 wherein said antibodies are selected from the group consisting of antibodies to Alzheimer's disease amyloid peptide, GFAP protein, DR-antigen and HIV antigen.

20. A method according to claim 18 wherein said antibody is an antibody to an antigen selected from the group consisting of Alzheimer's disease amyloid peptide, GFAP protein, DR-antigen and HIV antigen.

Fig. 2.

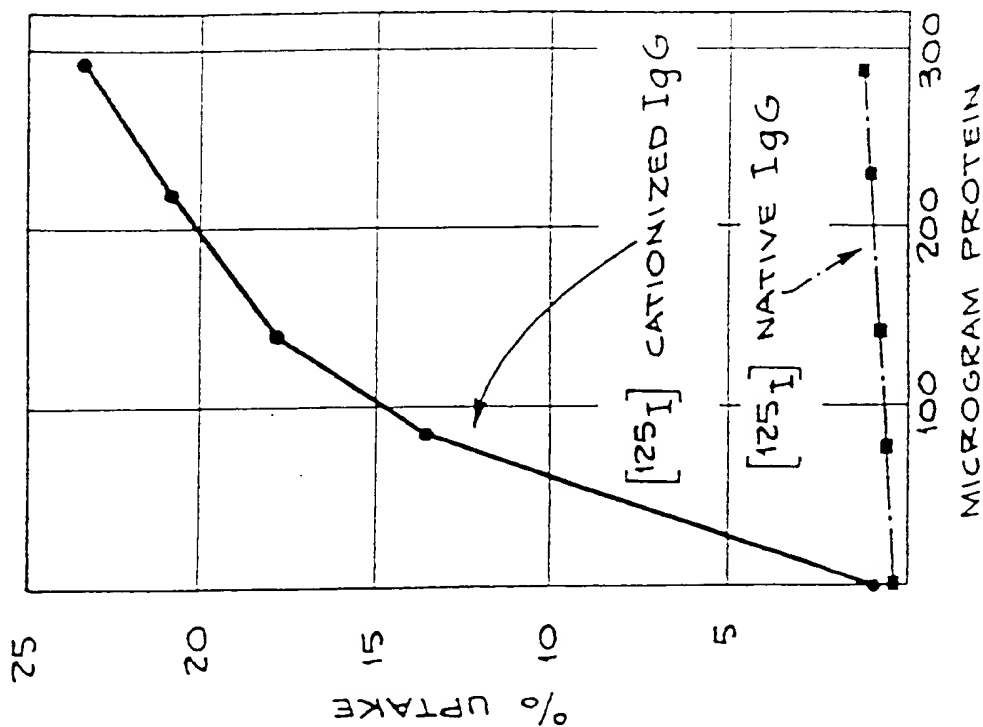
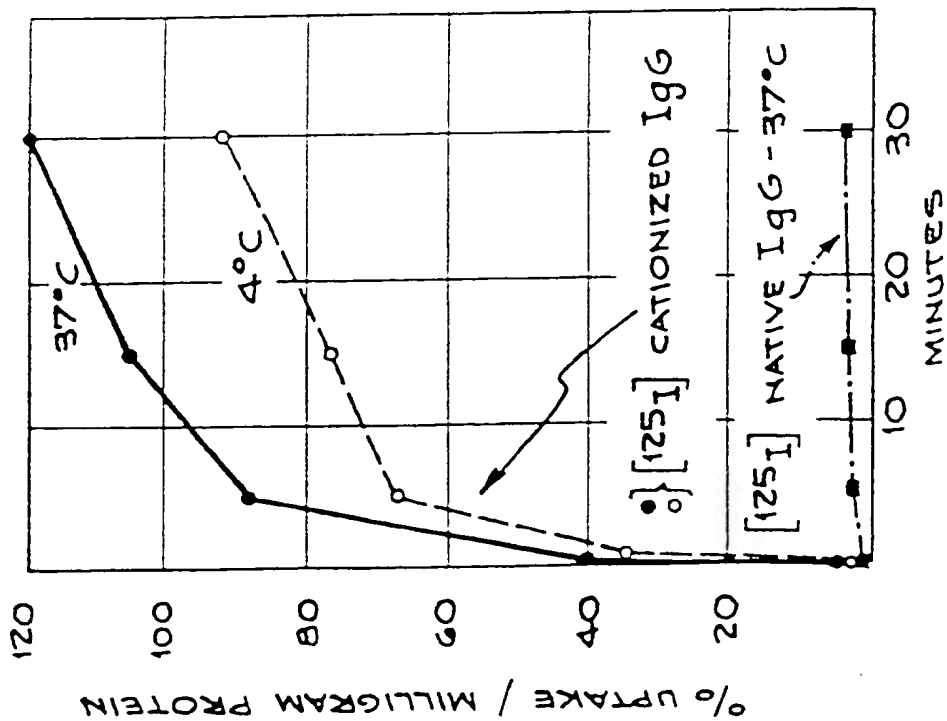
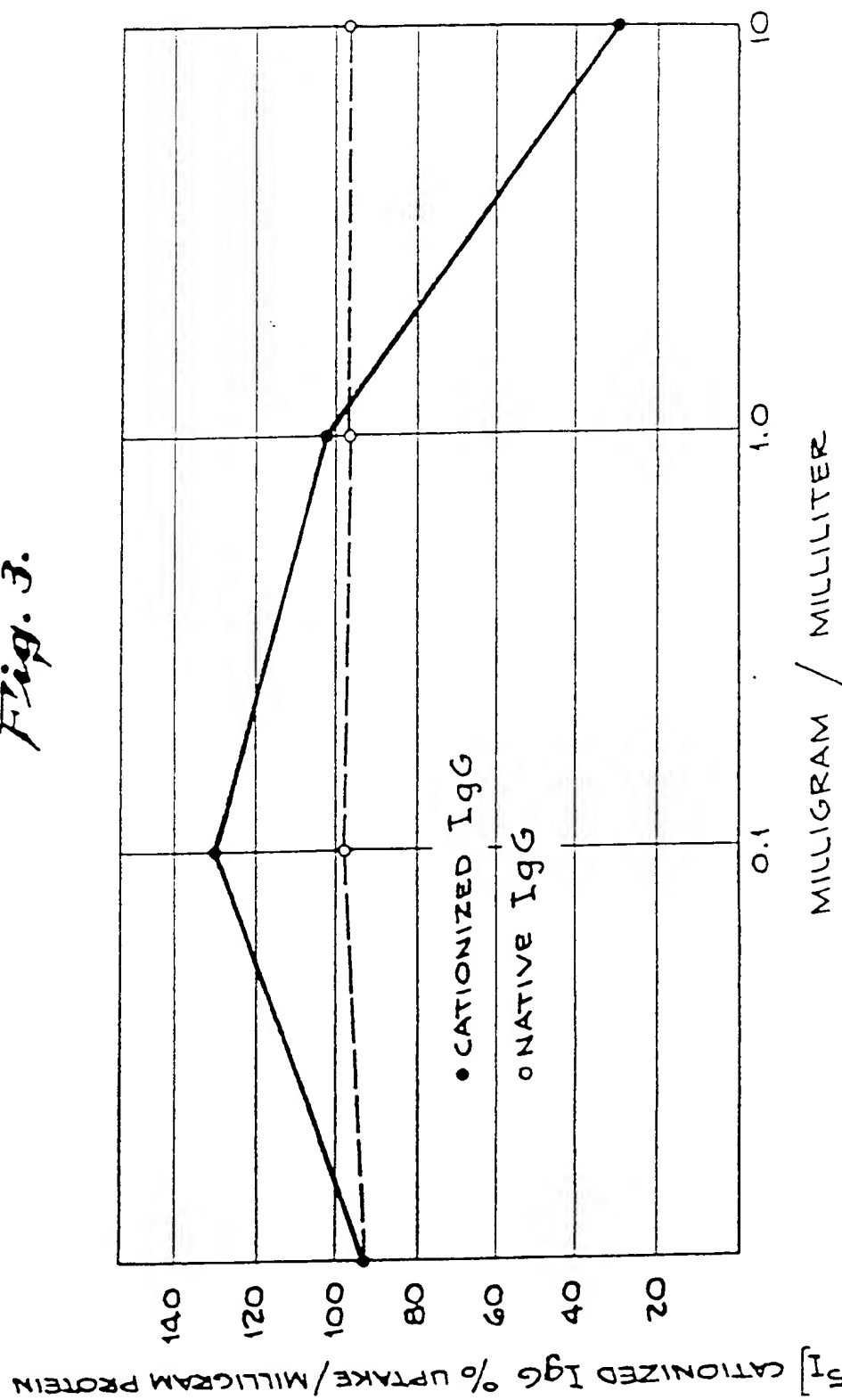


Fig. 1.



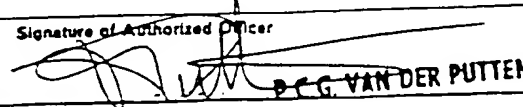
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Fig. 3.



INTERNATIONAL SEARCH REPORT

International Application No PCT/US 86/02760

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ¹ According to International Patent Classification (IPC) or to both National Classification and IPC A 61 K 49/00; C 07 K 15/00; A 61 K 39/395; //C 12 P 21/00 IPC ⁴ :		
II. FIELDS SEARCHED Minimum Documentation Searched ¹		
Classification System	Classification Symbols	
IPC ⁴	A 61 K; C 12 P	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ¹		
III. DOCUMENTS CONSIDERED TO BE RELEVANT¹		
Category [*]	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
A	US, A, 4118379 (R. SCHMIDTBERGER) 3 October 1978 see column 1, lines 7-12, line 55 - column 2, line 3; column 2, lines 28-40; claims 1-7,16 --	1-20
A	The Journal of Immunology, vol. 133, no. 6, December 1984 The American Ass. of Immunologists (US) B.H. Hahn et al.: "A public idiotypic determinant is present on spontaneous cationic IgG antibodies to DNA from mice of unrelated lupus prone strains" pages 3015-3019, see page 3015, abstract, page 3017, left-hand column, paragraph 1 - page 3018, left-hand column, paragraph 1 --	1,6
A	Chemical Abstracts, vol. 107, 1987, (Columbus, Ohio, US) W.M. Pardridge et al.: "High molecular weight Alzheimer's disease amyloid peptide immunoreactivity in human serum and CSF is an immunoglobulin G",	1,4,6,9
[*] Special categories of cited documents: 10 ¹¹ "A" document defining the general state of the art which is not considered to be of particular relevance ¹² "E" earlier document but published on or after the international filing date ¹³ "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) ¹⁴ "O" document referring to an oral disclosure, use, exhibition or other means ¹⁵ "P" document published prior to the international filing date but later than the priority date claimed ¹⁶ "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention ¹⁷ "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step ¹⁸ "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such docu- ments, such combination being obvious to a person skilled in the art ¹⁹ "Z" document member of the same patent family		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search 16th December 1988		Date of Mailing of this International Search Report 11 JAN 1989
International Searching Authority EUROPEAN PATENT OFFICE		Signature of Authorized Officer  P.C.G. VAN DER PUTTEN

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
	see page 524, abstract no. 37764f & Biochem. Biophys. Res. Commun. 1987, 145(1), 241-8. -----	

ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.

US 8302760
SA 24264

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 05/01/89
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